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Original Article

Prevalence and risk factors for carriage of multi-drug resistant *Staphylococci* in healthy cats and dogs

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We investigated the distribution of commensal staphylococcal species and determined the prevalence of multi-drug resistance in healthy cats and dogs. Risk factors associated with the carriage of multi-drug resistant strains were explored. Isolates from 256 dogs and 277 cats were identified at the species level using matrix-assisted laser desorption ionisation-time of flight mass spectrometry. The diversity of coagulase-negative *Staphylococci* (CNS) was high, with 22 species in dogs and 24 in cats. Multi-drug resistance was frequent (17%) and not always associated with the presence of the *mecA* gene. A stay in a veterinary clinic in the last year was associated with an increased risk of colonisation by multi-drug resistant *Staphylococci* (OR = 2.4, 95% CI: 1.1 ~ 5.2, *p* value LRT = 0.04). When identifying efficient control strategies against antibiotic resistance, the presence of mechanisms other than methicillin resistance and the possible role of CNS in the spread of resistance determinants should be considered.

Keywords: antibiotics, coagulase-negative, matrix-assisted laser desorption ionisation-time of flight, staphylococci

Introduction

Staphylococci resistant against methicillin and other antibiotics have frequently been reported in pets [5,27]. These microorganisms are opportunistic pathogens that may colonise the skin and mucosae of humans and other animals. *Staphylococcus* is currently divided into coagulase-positive and coagulase-negative species. The pathogenicity of coagulase-negative *Staphylococci* (CNS) has long been underestimated because they were associated with more chronic or subacute infections when compared to coagulase-positive *Staphylococci* (CPS) [20]. However, the etiological role of CNS in prosthesis and foreign body infections is increasingly being recognised in human medicine [12,13,23]. In pets, the pathogenic potential of these microorganisms has not yet been clearly recognized, although there have been some reports of infections related to methicillin-resistant CNS in cats and dogs [11,24].

Few studies have addressed the composition of staphylococcal populations on the mucosae of healthy cats and dogs [8,9]. Previous investigations of the staphylococcal species diversity in these animals have focused on clinical isolates [34], mainly CPS [17], or described the distribution of well defined antibiotic resistance within a limited number of staphylococcal species [1,38]. However, these studies were carried out before 2005, when *Staphylococcus* (*S.*) *pseudintermedius* had not yet been described. In fact, this species had probably been reported in all previous studies as *S. intermedius*, leading to confusion regarding its actual occurrence in pets [14,21,22,29,30]. *S. pseudintermedius* has recently been suggested as the most relevant and prevalent CPS dog coloniser, and there have been increasing reports on its pathogenicity and methicillin resistance [15].

To date, CNS strains in pets has been neglected; however, the recent development of new molecular techniques has allowed accurate identification of CNS [3,31], which will eventually lead to a better understanding of these bacterial species. Additional knowledge regarding CNS carriage in animals will be of benefit, because these bacteria might represent a pool of antibiotic resistance for CPS species. Indeed, horizontal gene transfer of staphylococcal chromosome cassette *mec* (SCC*mec*) between CPS and

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CNS species has been documented [19].

In the last decade, several authors have suggested that pets may be reservoirs of antibiotic resistant bacteria [18,25,26]. This assumption was mainly based on studies reporting antibiotic resistance in clinical CPS isolates from dogs and humans in close contact [28,37]. However, a clear picture of the distribution, diversity and multi-drug resistance of both CPS and CNS species in pets is lacking, as is the role of cats and dogs as reservoirs of antibiotic resistance.

The purpose of the present study was to gain insight into the distribution of commensal staphylococcal species of healthy cats and dogs and determine the occurrence of multi-drug resistance in both CNS and CPS. We also explored risk factors associated with the carriage of these microorganisms by pets.

Materials and Methods

Study design and settings

Samples were collected between March 2008 and December 2009 from four different Swiss cantons (Berne, Ticino, Vaud and Zurich). Only healthy pets with no overt acute disease at the time of sample collection were enrolled in the study. The pets either lived in or visited nursing homes for pet-therapy or lived in households. The selection strategy differed between community and nursing homes. Pets in the community were included in the study based on convenience sampling in households (n = 196) in four Swiss cantons representing the northern, southern, central and western part of Switzerland. Additional pets (n = 239) were recruited from cats and dogs visiting a total of 12 veterinary practices in the same regions for routine vaccinations. Nursing homes were selected by two-stage random cluster sampling from an exhaustive list of nursing homes located in the four Swiss cantons as reported in [16]. In randomly selected nursing homes, all pets matching the inclusion criteria and present at the time of sample collection (n = 98) were enrolled in the study. Informed written consent was obtained from all pet owners prior to the start of the study, and the investigation received the approval for animal experimentation from the Cantonal and Swiss Federal Veterinary Offices (authorisation reference No. 01/2008-02/2008).

Sample collection

Nasal and ear swab samples were collected using cotton swabs (Amies agar gel 108C and 110C; Copan, Italy) that had been soaked in a physiological 9% NaCl solution. For collection, a swab was introduced 1～2 cm in the nostril, while a second swab was introduced as deeply as possible in the ear channel of each animal. The collected samples were then stored in transport medium at room temperature and analyzed for the presence of *Staphylococci* within 24～48 h of collection. A questionnaire collecting information regarding the demographic and health status of the pets was filled in by the owners (available on request).

Sample analyses

Both swabs were streaked onto Mannitol Salt Agar (Chapman 2 - MSA 2; bioMérieux, France), after which they were incubated for 48 h at 37°C, enriched in MRSA broth supplemented with 6 μg of oxacilllin (48 h at 37°C) and cultured on Gelose ChromID *S. aureus* (SAID; bioMérieux) for 48 h at 37°C. All morphologically different colonies were isolated and catalase positive, Gram positive coccal bacteria were frozen in skim milk at −80°C until further analyses.

Isolates were grown on blood agar for 24 h and then identified by matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS) using an Axima Confidence spectrometer (Shimadzu-Biotech, Japan) in positive linear mode (m/z = 2,000 to 20,000) [10]. The identity of isolates that could not be identified by MALDI-TOF MS (24%) was confirmed by sequencing of the amplified partial *rpoB* gene [31].

Phenotypic antibiotic resistance to 24 different drugs was assessed by the Kirby-Bauer method on Mueller-Hinton blood agar (MHS2; bioMérieux). The following antibiotics were tested: penicillin (10 units), ampicillin (10 μg), oxacillin (1 μg), cefazolin (30 μg), gentamicin (10 μg), tetracycline (30 μg), erythromycin (15 μg), clindamycin (2 μg), vancomycin (30 μg), trimethoprim-sulfamethoxazole (1.25 + 23.75 μg), ciprofloxacin (5 μg), amoxicillin and clavulanic acid (20 + 10 μg), ceftazidim (30 μg), imipenem (10 μg), tobramycin (10 μg), fusidic acid (10 μg), rifampicin (30 μg), chloramphenicol (30 μg), cefoxitin (30 μg), kanamycin (30 μg), doxycyclin (30 μg), mupirocin (5 μg), linezolid (30 μg) and quinopristin-dalfopristin (15 μg). An inducible clindamycin resistance test ("D-zone" test) was also carried out for all isolates. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [4,32], and intermediate results were classified as resistant. Multi-drug resistance (MDR) was defined as resistance to at least three drugs belonging to three different antibiotic classes [16]. Additionally, the presence of the *mec*A gene, which confers methicillin resistance, was investigated by polymerase chain reaction (PCR) on all isolates that showed phenotypic resistance to oxacillin [6,7]. We considered isolates from the same animal as being different strains if they belonged to different staphylococcal species or their phenotypic antibiotic resistance profiles differed.

Statistical analyses

Sample size calculation was based on the assumption that 5% of pets carried at least one MDR staphylococcal strain and that the intra-class correlation coefficient (rho) in these

settings was 0.15. We used the cluster sample equation developed by Bennett *et al*. [2] for all sample size calculations. Assuming that each nursing home with pets owned or was visited by three animals on average, sample collection in 42 different nursing homes would have provided 126 pets. Accordingly, the expected precision for the prevalence estimate of MDR in pets would have a standard error of 2.2%, and a 95% confidence interval (CI) = 0.68～9.3%.

The characteristics of the cats and dogs were compared to check for consistent differences in the demographics and health status of the different populations sampled. A Chi-square test (Fisher's exact test when expected observations < 5) and 95% CI were used for this comparison. We reported the prevalence of *Staphylococci* and MDR *Staphylococci* and the distribution of antibiotic resistance among different staphylococcal species together with the median number of resistances to different antibiotic classes. Univariable logistic regression models with MDR staphylococcal carriage status of the pet as the outcome variable of interest were applied to explore risk factors. Unadjusted odds ratios (OR with 95% CI) were calculated as a measure of association. Statistical significance of each explanatory variable was determined

Table 1. Demographics of investigated cats and dogs

Characteristics	Nursing home (N = 98)			Community (N = 435)		
	n/N	%*	95% CI	n/N	%*	95% CI
Cats	53/98	54	44～63	224/435	51	47～56
Female	63/98	64	54～73	226/434	52	47～57
Sterilized	80/98	82	73～88	306/433	71	66～75
Age						
< 3 years	19/98	19	12～27	156/435	36	31～40
3～10 years	56/98	57	47～67	189/435	43	39～48
>10 years	23/98	23	15～32	90/435	21	17～24
Cantons						
Berne	32/98	33	23～42	95/435	22	18～26
Ticino	26/98	27	18～35	140/435	32	28～37
Vaud	20/98	20	12～28	107/435	25	21～29
Zurich	20/98	20	12～28	93/435	21	18～25
Visited veterinary clinics in the last year	3/97	3	1～9	28/431	7	5～9
Stayed in an animal home in the last year	8/98	8	4～15	22/434	5	3～8
Pyoderma in the last year	3/96	3	1～9	22/423	5	3～8
Urinary infections in the last year	4/93	4	2～11	17/421	4	3～6
Otitis in the last year	3/93	3	1～10	39/425	9	7～12
Antibiotic treatment in the last 3 months	5/94	5	2～12	59/429	14	11～17
Immunosuppressant treatment in the last 3 months	3/92	3	1～10	20/423	5	3～7

*Proportions. CI: confidence interval.

Table 2. Proportion of staphylococcal and MDR staphylococcal carriage in cats and dogs

	Staphylococcal carriage			Multi-drug resistance carriage					
				Staphylococci			CPS	CNS	CPS & CNS
	n/N	%*	95% CI	n/N	%*	95% CI	n/N	n/N	n/N
Total	320/533	60.0	55.8～64.1	92/533	17.3	14.3～20.7	21/92	67/92	4/92
Cats	164/277	59.2	53.3～64.8	41/277	14.8	11.0～19.5	1/41	39/41	1/41
Dogs	156/256	60.9	54.8～66.7	51/256	20.0	15.5～25.4	20/51	28/51	3/51

*Proportions. CPS: coagulase-positive staphylococci, CNS: coagulase-negative staphylococci.

by a likelihood-ratio test (LRT). We included in a multivariable model all variables with LRT p values ≤ 0.2 from the univariable analysis. All statistical analyses were performed using STATA 9.0 (Stata Corporation, USA).

Results

Demographics and staphylococcal carriage in pets

We collected samples from 533 healthy pets (277 cats and 256 dogs), 98 of which lived in or visited nursing homes at least once a week and 435 that lived in the community. The demographics of the two populations studied are reported in Table 1. Parameters such as sex, age, sterilisation, otitis in the last year, and antibiotic treatment showed different distributions between the nursing home and community settings, but the 95% CI estimates of these parameters overlapped (Table 1). We did not carry out stratified analyses of the samples because the overall frequencies of MDR in nursing homes (15/98) and in the community (76/435) did not differ significantly ($\chi^2 = 0.27$, $p = 0.6$).

Staphylococci were detected in 60% (320/533) of pets, and 17% (92/533) of all animals carried at least one MDR strain. There were no significant differences in MDR carriage between pet species [14.8% (95% CI: 11.0 ~ 19.5)

Table 3. *Staphylococci* in nostrils and ears of dogs (N = 256) and cats (N = 277) and their antibiotic resistance profiles

Identified isolates	Dogs						Cats					
	MDR isolates*		Max. AB†		*mecA*‡		MDR isolates*		Max. AB†		*mecA*‡	
	Nostril	Ear	Nostril	Ear	Nostril	Ear	Nostril	Ear	Nostril	Ear	Nostril	Ear
Coagulase positive	15/69	9/29	6	6	0/69	0/29	0/17	2/5	1	5	0/17	0/5
S. aureus	0/10	0/3	2	2	0/10	0/3	0/11	0/3	1	1	0/11	0/3
S. pseudintermedius	15/59	9/26	6	6	0/59	0/26	0/6	2/2	0	5	0/6	0/2
Coagulase negative	22/97	14/75	8	8	8/97	3/75	23/132	19/126	7	7	3/132	4/126
S. arlettae	1/1	−	3	-	0/1	−	1/1	−	6	−	0/1	−
S. auricularis	0/1	0/1	0	0	0/1	0/1	0/4	1/10	0	4	0/4	0/10
S. capitis	0/3	0/1	0	0	0/3	0/1	1/1	1/6	3	3	0/1	0/6
S. caprae	−	−	−	−	−	−	−	0/1	−	1	−	0/1
S. caprae/capitis	0/5	0/3	2	1	0/5	0/3	0/4	0/5	2	1	0/4	0/5
S. cohnii	2/4	0/1	5	1	0/4	0/1	0/2	1/3	1	4	0/2	0/3
S. devriesei	−	0/3	−	0	−	0/3	−	−	−	−	−	−
S. epidermidis	2/15	1/12	7	3	2/15	1/12	5/16	4/20	4	7	1/16	4/20
S. equorum	2/9	0/3	4	0	1/9	0/3	1/11	0/10	7	2	0/11	0/10
S. felis	−	−	−	−	−	−	4/41	2/28	3	3	0/41	0/28
S. haemolyticus	2/8	2/5	8	8	2/8	0/5	2/5	0/1	3	1	0/5	0/1
S. hominis	2/9	1/11	3	3	2/9	1/11	1/5	0/4	5	1	1/5	0/4
S. kloosi	−	0/1	−	1	−	0/1	−	−	−	−	−	−
S. lentus	−	1/1	−	3	−	0/1	1/3	1/1	4	3	0/3	0/1
S. lugdunensis	−	0/1	−	0	−	0/1	−	1/2	−	3	−	0/2
S. nepalensis	−	−	−	−	−	−	1/2	−	4	−	0/2	−
S. pasteuri	0/2	0/1	1	1	0/2	0/1	−	0/1	−	2	−	0/1
S. pettenkoferi	0/2	−	0	−	0/2	−	0/4	0/4	0	0	0/4	0/4
S. saprophyticus	0/4	1/5	2	3	0/4	0/5	0/2	1/4	2	3	0/2	0/4
S. sciuri	6/6	1/1	4	4	1/6	0/1	2/5	1/3	3	3	1/5	0/3
S. simulans	0/1	−	1	−	0/1	−	0/3	1/8	2	3	0/3	0/8
S. succinus	0/3	0/1	2	0	0/3	0/1	−	0/1	−	0	−	0/1
S. vitulinus	0/6	0/2	1	1	0/6	0/2	0/1	−	1	−	0/1	−
S. warneri	2/9	5/13	3	5	0/9	1/13	1/12	2/6	4	4	0/12	0/6
S. xylosus	3/9	2/9	4	4	0/9	0/9	3/10	3/8	4	3	0/10	0/8
Other staphylococci												
S. schleiferi subsp.	−	−	−	−	−	−	0/1	0/1	0	0	0/1	0/1
Staphylococcus spp.	2/10	0/4	3	1	0/10	0/4	0/3	1/15	2	4	0/3	0/15

*Number of multi-drug resistant isolates over the total number of isolates for a given species or group. †Maximum number of different resistant antibiotic classes. ‡Number of strains carrying the gene encoding methicillin resistance over the total number of isolates for a given species or group.

in cats and 20.0% (95% CI: 15.5 ~ 25.4) in dogs; $\chi^2 = 2.1$, $p = 0.14$] (Table 2). In cats, most CNS were MDR (39/41), whereas the proportion of MDR in CPS was small (1/41). Conversely, MDR CPS (20/51) and MDR CNS (28/51) carriage was almost equal in dogs (Table 2). Additionally, we observed species-specific differences ($\chi^2 = 63.69$, $p < 0.001$) in the proportion of *S. pseudintermedius* carriage, with 27% (70/256) of dogs and 3% of cats (8/277) harbouring this species. No difference in *S. aureus* carriage was observed between the two pet species (13/256 dogs and 14/277 cats, respectively).

Staphylococcal isolates

We isolated 284 staphylococcal strains (176 from the nostrils and 108 from the ears) from dogs and 300 (153 from the nostrils and 147 from the ears) from cats (Table 3). We were able to identify 94.5% (552/584) of all isolates at the species level. Two *S. schleiferi* isolates from two cats were identified only at the species level. CNS species accounted for 60% (172/284) of all isolates in dogs and 86% (258/300) in cats (Table 3).

In cats, the total number of CPS strains was lower (22/300) than in dogs (98/284). Among the CPS strains, *S. pseudintermedius* was more frequently isolated from dogs [(85/98), 87%] than from cats [(8/22), 36%], whereas *S. aureus* was more frequent in cats [(14/22), 63%] than dogs [(13/98), 13%]. No other CPS were isolated.

The diversity of CNS was high, with 22 different species in dogs and 24 in cats (Table 3). *S. felis* was only isolated from cats, in particular from their nostrils, and it represented 31% of all CNS isolates (41/132). Other CNS recovered in relevant proportions from both pets were *S. epidermidis*, *S. warneri*, *S. hominis*, *S. xylosus* and *S. equorum* (Table 3).

Antibiotic resistance

The *mecA* gene was present in 6% (11/172) of dog and 3% (7/258) of cat isolates. We did not recover any MDR *S. aureus* (Table 3). MDR, with a few strains showing resistance to eight different antibiotic classes, was detected in bacteria at proportions of 21% (36/172) in dogs and 16% (42/258) in cats. MDR was observed in *S. pseudintermedius* isolated from both pet species with resistance to up to six different antibiotic classes, but no methicillin resistance was seen (Table 3).

About 50% of all isolates in dogs and 30% in cats showed phenotypic resistance to penicillin and ampicillin (Table 4). Fusidic acid and erythromycin resistance were detected in 31% and 25% of dog and 28% and 19% of cat isolates, respectively. Additionally, 15% of all strains isolated from dogs were resistant to tetracycline and 11% to kanamycin. Clindamycin resistance was reported from 16% of dog and 15% of cat isolates (Table 4).

Exploratory analysis of risk factors

Univariable exploratory analysis revealed that a stay in a veterinary clinic in the last year was associated with increased risk of colonisation by MDR *Staphylococci* (OR = 2.4, 95% CI: 1.1 ~ 5.2, *p* value LRT = 0.04; Table 5). We included species, canton, stay in veterinary clinic in the last year, and antibiotic treatment in the last 3 months in the multivariable analysis. No missing data for these variables was observed for all 465 records. When accounting for other variables, we observed an influence of the cantons (geographic origin) on the carriage of MDR *Staphylococci* (*p* value LRT = 0.02). Additionally, cats had a lower risk of being carriers of MDR *Staphylococci*, whereas a stay in a veterinary clinic in the last year and antibiotic treatment in the last 3 months were associated with a higher risk, although these differences were not statistically significant (Table 5).

Table 4. *In vitro* antibiotic resistance of the isolates investigated

	Isolates from dogs (N = 284)	Isolates from cats (N = 300)
	n (%)	n (%)
Penicillin	140 (49)	90 (30)
Ampicillin	131 (46)	77 (26)
Oxacillin	13 (5)	7 (2)
Ceftazidim	13 (5)	8 (3)
Cefoxitin	9 (3)	7 (2)
Cefazolin	1 (0.3)	0
Co-amoxicillin	1 (0.3)	1 (0.3)
Imipenem	0	0
Kanamycin	31 (11)	4 (1)
Gentamicin	5 (2)	1 (0.3)
Tobramycin	1 (0.3)	0
Tetracycline	44 (15)	17 (6)
Doxycyclin	35 (12)	12 (4)
Erythromycin	72 (25)	58 (19)
Clindamycin	45 (16)	39 (15)
Vancomycin	0	0
Ciprofloxacin	4 (1)	5 (2)
Trimethoprim-sulfamethoxazole	10 (3)	4 (1)
Rifampicin	0	0
Chloramphenicol	22 (8)	4 (1)
Linezolid	0	0
Quinopristin-dalfopristin	8 (3)	10 (3)
Fusidic acid	87 (31)	85 (28)
Mupirocin	0	1 (0.3)

Table 5. Risk factors for cats and dogs to be carriers of MDR *Staphylococci*

Variable level		MDR	Univariable analysis			Multivariable model		
			OR		LRT	OR[†]		LRT
	N	n (%)		95% CI	*p* value*		95% CI	*p* value*
Origin								
Nursing homes	98	15 (15)	Baseline					
Community setting	434	76 (18)	1.1	0.6 ~ 2.0	0.6	Not included		
Species								
Dog	255	50 (20)	Baseline			Baseline		
Cat	277	41 (15)	0.7	0.4 ~ 1.1	0.1	0.7	0.4 ~ 1.1	0.1
Sex								
Male	243	42 (17)	Baseline					
Female	288	49 (17)	1.0	0.6 ~ 1.5	0.9	Not included		
Age								
0 ~ 3 years	175	36 (21)	Baseline					
3 ~ 10 years	245	40 (16)	0.8	0.5 ~ 1.2				
10 ~ 20 years	112	15 (13)	0.6	0.3 ~ 1.2	0.3	Not included		
Sterilised								
No	145	27 (19)	Baseline					
Yes	385	64 (17)	0.9	0.5 ~ 1.4	0.6	Not included		
Canton								
Bern	126	20 (16)	Baseline			Baseline		
Ticino	166	20 (12)	0.7	0.4 ~ 1.4		0.6	0.3 ~ 1.2	
Vaud	127	27 (21)	1.4	0.8 ~ 2.7		1.5	0.8 ~ 3.0	
Zurich	113	24 (21)	1.4	0.7 ~ 2.8	0.1	1.5	0.8 ~ 3.0	0.02
Stayed in veterinary clinic in the last year								
No	496	81 (16)	Baseline			Baseline		
Yes	31	10 (32)	2.4	1.1 ~ 5.4	0.04	1.3	0.4 ~ 3.8	0.6
Stayed in animal home in the last year								
No	501	84 (17)	Baseline					
Yes	30	7 (23)	1.5	0.6 ~ 3.6	0.4	Not included		
Pyoderma in the last year								
No	493	85 (17)	Baseline					
Yes	25	5 (20)	1.2	0.4 ~ 3.3	0.7	Not included		
Urinary infection in the last year								
No	492	88 (18)	Baseline					
Yes	21	2 (10)	0.5	0.1 ~ 2.1	0.3	Not included		
Otitis in the last year								
No	475	84 (18)	Baseline					
Yes	42	7 (17)	0.9	0.4 ~ 2.2	0.9	Not included		
Antibiotic treatment in the last 3 months								
No	458	73 (16)	Baseline			Baseline		
Yes	64	15 (23)	1.6	0.9 ~ 3.0	0.1	1.3	0.6 ~ 2.8	0.4
Immunosuppressant in the last 3 months								
No	491	79 (16)	Baseline					
Yes	23	6 (26)	1.8	0.7 ~ 4.8	0.24	Not included		

*p value considered statistically significant if ≤ 0.05. MDR: multi-drug resistance, OR: odds ratio, LRT: likelihood-ratio test.

Discussion

This study provides detailed information on staphylococcal carriage in healthy cats and dogs and on drug resistance of these bacteria to different antibiotic classes for the first time since the description of *S. pseudintermedius*. We showed that *S. pseudintermedius* was recovered from the mucosae of healthy dogs more frequently than from those of healthy cats. Previous hospitalisation (at least one night in a veterinary clinic) was a risk factor for the carriage of MDR *Staphylococci* in pets using the univariable approach. The multivariable model

showed that geographical distribution of the animals in the four cantons had an influence on the carriage of MDR staphylococci, which might reflect different pet health care and prescription practices of veterinarians in different regions of Switzerland.

Identification of the *Staphylococci* was carried out by MALDI-TOF MS, which provides reliable and rapid identification of the taxa in the *S. intermedius* group (*S. delphini*, *S. intermedius* and *S. pseudintermedius*) [10]. Previous investigations of the staphylococcal population of the mucosae of cats and dogs were based on phenotypic characterisation of the isolates, which may have led to misidentification of some closely related staphylococcal species [22,39].

We isolated MDR staphylococcal strains from healthy cats and dogs; however, MDR was not always associated with the presence of the *mecA* gene. In this study, resistance of strains to different antibiotic classes ranged from very low proportions (*e.g.*, 1～2% resistance to ciprofloxacin in cats and dogs) to high values (11%) for kanamycin resistance in dogs. Methicillin resistance is of particular interest, because it confers resistance to all beta-lactams and is also often linked to resistance to other antibiotic classes; however, other resistances are also relevant in clinical settings, and infections resulting from MDR opportunistic pathogens are a critical problem to clinicians because they limit the choice of active antibiotic treatments [36].

It should be noted that our study has some limitations. Specifically, the exploratory analysis of risk factors was carried out by combining all staphylococcal species and information on pet-therapy animals as well as household pets, even though the risk associated with the carriage of MDR *Staphylococci* belonging to several species might differ between groups. This approach was necessary because the numbers for given combinations of investigated risk factors and animals carrying different MDR staphylococcal species were small. Pet management factors in the three months preceding the study were reported by the owners; therefore, a recall bias might be present. However, we do not consider this potential bias to be important because one can reasonably expect pet owners to recall whether or not a pet had visited a veterinary clinic during the preceding three months. We did not collect data on the number of different antibiotic treatments and the length of treatments, and the analysis of these data might have revealed other risk factors. In addition, we defined MDR as resistance of a strain to at least three antibiotics of different classes. Official guidelines (*e.g.*, CLSI and EUCAST) lack a clear and standard criteria to define a staphylococcal strain as MDR, which reduces the possibility of carrying out meaningful comparisons with published data [16]. Despite the limitations of an exploratory univariable approach, our results confirm findings from published studies regarding factors associated with the carriage of MDR *Staphylococci* in pets, and in particular, the importance of previous hospitalisation, which was already reported as a risk factor for acquisition of both MRSA and MRSP in pets [33,35].

Our study has shown that carriage of multi-drug resistant *Staphylococci* in healthy cats and dogs is common; thus, clinical therapy guidelines would benefit from an approach that is not only focused on methicillin resistance, neglecting the presence of other resistances. The monitoring of antibiotics use in veterinary clinics could provide an overview of possible future trends of antibiotic resistance in pets. In veterinary medicine, further studies investigating the dissemination of antibiotic resistance determinants would benefit from considering the possible role of reservoirs of CNS in their spread.

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